
CHROMATOGRAM**Retention time:** 11**Limit of detection:** 1000 ng

OTHER SUBSTANCES**Extracted:** chenodiol, bile acids, deoxycholic acid

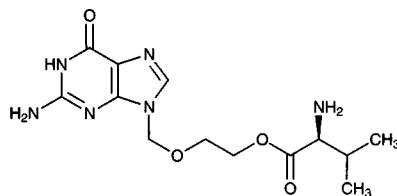
KEY WORDS

SPE

REFERENCE

Batta,A.K.; Shefer,S.; Batta,M.; Salen,G. Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography, *J.Lipid Res.*, **1985**, 26, 690-698.

Valacyclovir

Molecular formula: C₁₃H₂₀N₆O₄**Molecular weight:** 324.34**CAS Registry No.:** 124832-26-4, 124832-27-5 (HCl)**Merck Index:** 10039

SAMPLE**Matrix:** tissue

Sample preparation: Blend 20% intestinal tissue in Ringers buffer at high speed for 2 min. Centrifuge the homogenate at 12000 g for 15 min at 4°. Remove a 50 µL aliquot of the supernatant, add 450 µL stopping solution. Centrifuge at 12000 g for 5 min at 4°, inject an aliquot of the supernatant. (Stopping solution was an ice cold mixture of 200 µL MeOH and 200 µL pH 6.5 buffer. Buffer was 15 mM 2-[N-morpholino]-ethanesulfonic acid (MES), 130 mM NaCl, 5 mM KCl, and 0.01% PEG-3350.)

HPLC VARIABLES**Guard column:** 20 mm long Supelguard LC-18S**Column:** 250 × 4.6 Supelcosil LC-18S

Mobile phase: Water:buffer 20:80 (Buffer was MeOH:100 mM potassium phosphate monobasic 25:75, adjusted to pH 6.7 with 1 M NaOH. Water:buffer ratio may be adjusted to ensure separation of other compounds.)

Flow rate: 1**Detector:** UV 252

CHROMATOGRAM**Limit of quantitation:** 1 µM

OTHER SUBSTANCES

Simultaneous: p-aminohippuric acid sodium salt, amoxicillin, ampicillin, cefadroxil, cephadrine, formycin B, quinine, stavudine, thymidine, valine

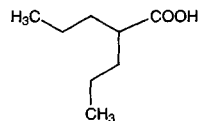
KEY WORDS

rat; intestine

REFERENCE

Sinko,P.J.; Balimane,P.V. Carrier-mediated intestinal absorption of valacyclovir, the L-valyl ester prodrug of acyclovir: 1. Interactions with peptides, organic anions and organic cations in rats, *Biopharm.Drug Dispos.*, **1998**, 19, 209-217.

Valproic acid



Molecular formula: $C_8H_{16}O_2$

Molecular weight: 144.21

CAS Registry No.: 99-66-1, 1069-66-5 (sodium salt), 76584-70-8 (divalproex, 1:1 mixture of valproic acid and sodium valproate)

Merck Index: 10049

SAMPLE

Matrix: blood

Sample preparation: Mix 20 μ L serum with 100 μ g/mL IS, add 400 μ L 300 mM pH 2.5 citrate/phosphate buffer, stir the mixture, add it to a 350 mg Extrelut SPE cartridge, adsorb for 20 min, elute with 12 mL chloroform. Add 25 μ L 0.1% NaOH in MeOH to the eluate and concentrate it in vacuum. Mix the residue with 25 μ L each 2% thionyl chloride in chloroform and 8% triethylamine in chloroform, stir at room temperature for 30 s, add 150 μ L 10 mg/mL 9-aminophenanthrene in chloroform, stir at -10 to -5° for 90 min. Inject a 10 μ L aliquot of the reaction mixture directly. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 150 \times 6.5 μ m Shimpack CLC-ODS

Mobile phase: MeCN:MeOH:water 11:23:6

Column temperature: 40

Flow rate: 1

Injection volume: 10

Detector: F ex 303 em 376

CHROMATOGRAM

Retention time: 15.2

Internal standard: cyclohexane carboxylic acid (10.8)

Limit of detection: 9.4 pg

Limit of quantitation: 5 μ g/mL

OTHER SUBSTANCES

Noninterfering: phenobarbital, phenytoin, carbamazepine

KEY WORDS

serum; derivatization; SPE

REFERENCE

Nakajima, M.; Sato, A.; Shimada, K. Determination of serum valproate by high-performance liquid chromatography using fluorescence labeling with 9-aminophenanthrene, *Anal. Sci.*, **1988**, *4*, 385-388.

SAMPLE

Matrix: blood

Sample preparation: Add 200 μ L 2 μ g/mL thymol in MeCN to 200 μ L serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 Resolve C18-5 (Waters)

Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60

Column temperature: 30

Flow rate: 0.7

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 13.5

Internal standard: thymol (18.5)

OTHER SUBSTANCES

Extracted: ethosuximide, primidone, phenobarbital, phenytoin, carbamazepine

KEY WORDS

plasma

REFERENCE

Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE

Matrix: formulations

Sample preparation: Capsules. Dissolve 10 broken capsules in 200 mL acetone. Remove a 20 mL aliquot and make it up to 100 mL with acetone:water 45:55. Remove a 1 mL aliquot and make it up to 10 mL with acetone, mix. Remove a 1 mL aliquot and add it to 1 mL IS solution, add 50 μ L 12.8 mg/mL 2-bromoacetophenone (phenacyl bromide) in acetone, add 50 μ L 10 mg/mL triethylamine in acetone, mix with gentle swirling, heat at 50° for 2 h, cool to room temperature, inject an aliquot. Syrup. Dilute 5 mL syrup to 100 mL with acetone:water 45:55. Remove a 1 mL aliquot and make it up to 10 mL with acetone, mix. Remove a 1 mL aliquot and add it to 1 mL IS solution, add 50 μ L 12.8 mg/mL 2-bromoacetophenone (phenacyl bromide) in acetone, add 50 μ L 10 mg/mL triethylamine in acetone, mix with gentle swirling, heat at 50° for 2 h, cool to room temperature, inject an aliquot. Tablets. Weigh out powdered tablets equivalent to 250 mg valproic acid, add 50 mL acetone:water 45:55, sonicate for 10 min, make up to 100 mL with acetone:water 45:55, sonicate for 10 min, mix, centrifuge an aliquot of the suspension at 4000 rpm for 5 min. Remove a 1 mL aliquot of the supernatant and make it up to 10 mL with acetone, mix. Remove a 1 mL aliquot and add it to 1 mL IS solution, add 50 μ L 12.8 mg/mL 2-bromoacetophenone (phenacyl bromide) in acetone, add 50 μ L 10 mg/mL triethylamine in acetone, mix with gentle swirling, heat at 50° for 2 h, cool to room temperature, inject an aliquot. (Prepare IS solution by diluting a 400 μ g/mL solution of sodium caproate in acetone:water 45:55 with an equal volume of acetone.)

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Microsorb-MV C18

Mobile phase: MeCN:MeOH:water 50:20:30

Flow rate: 2

Injection volume: 50

Detector: UV 245

CHROMATOGRAM

Retention time: 8.5

Internal standard: caproic acid (4.5)

KEY WORDS

derivatization; capsules; syrup; tablets

REFERENCE

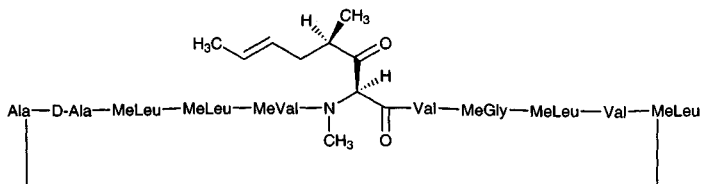
Lau-Cam,C.A.; Roos,R.W. HPLC method with precolumn phenacylation for the assay of valproic acid and its salts in pharmaceutical dosage forms, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2075–2087.

Valspodar

Molecular formula: C₆₃H₁₁₁N₁₁O₁₂

Molecular weight: 1214.65

CAS Registry No.: 121584-18-7



SAMPLE

Matrix: blood

Sample preparation: Mix whole blood with pH 9.0 borate buffer and saturated aqueous NaCl, add MTBE:ethyl acetate 50:50, extract, evaporated the extract under vacuum, reconstitute the residue in MeCN:water 40:60, inject an aliquot.

HPLC VARIABLES

Column: phenyl-bonded silica gel

Mobile phase: MeCN:water:MTBE 45:40:15

Column temperature: 70

Detector: UV 210

CHROMATOGRAM

Limit of quantitation: 30 ng/mL

KEY WORDS

pharmacokinetics; whole blood;

REFERENCE

Mueller,E.A.; Kovarik,J.M.; üresin,Y.; Preisig-Flückiger,S.S.; Hensel,S.; Lücker,P.W.; Holt,B. Optimizing the absorption of valspodar, a P-glycoprotein modulator, PartI: Selecting an oral formulation and exploring its clinical pharmacokinetics/dynamics, *J.Clin.Pharmacol.*, **1997**, 37, 1001-1008.

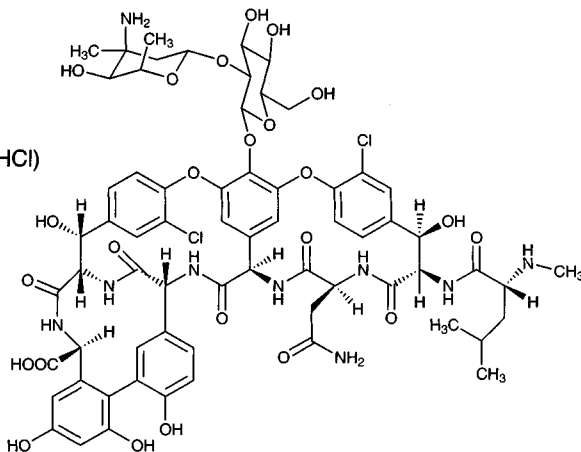
Vancomycin

Molecular formula: $C_{66}H_{75}Cl_2N_9O_{24}$

Molecular weight: 1449.27

CAS Registry No.: 1404-90-6, 1404-93-9 (HCl)

Merck Index: 10066



SAMPLE

Matrix: blood

Sample preparation: Condition a 800 μ L 500 mg Sep-Pak Vac 3cc C18 SPE cartridge twice with 800 μ L MeOH and with 800 μ L water. 200 μ L Serum + 200 μ L water + 50 μ L 100 mg/mL IS, vortex. Add to the SPE cartridge. Wash twice with 800 μ L water. Elute twice with 400 μ L MeCN:50 mM KH_2PO_4 70:30 and twice with 400 μ L MeCN:water 50:50. Evaporate the eluate under a stream of nitrogen at 50° for 10 min. Cool, centrifuge at 1450-1475 g for 4 min, inject a 50 μ L aliquot of the supernatant.

HPLC VARIABLES

Guard column: KGCQ-324C (YMC, Wilmington, NC)

Column: 250 \times 4.6 5 μ m YMC pack ODS-AQ (YMC, Wilmington, NC)

Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 6 phosphate buffer 5:4:91. B was MeCN:MeOH:50 mM pH 6 phosphate buffer 8:8:84. A:B 100:0 for 2 min, to 0:100 over 9 min, maintain at 0:100 for 14 min, to 100:0 over 5 min, maintain at 100:0 for 5 min (Prepare buffer as follows. Dissolve 11.94 g KH_2PO_4 and 2.14 g K_2HPO_4 in 2 L water.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 210

CHROMATOGRAM

Retention time: 18

Internal standard: cefazolin (27)

Limit of quantitation: 1 μ g/mL

OTHER SUBSTANCES

Extracted: degradation products

Simultaneous: acetaminophen, salicylates, theophylline

KEY WORDS

serum; SPE

REFERENCE

Backes,D.W.; Aboleneen,H.I.; Simpson,J.A. Quantitation of vancomycin and its crystalline degradation product (CDP-1) in human serum by high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1281-1287.

SAMPLE

Matrix: blood

Sample preparation: 50 μ L Serum + 9 μ L 125 μ g/mL trimethoprim + 75 μ L acetone:10% trichloroacetic acid 1:2, vortex for 5 s, centrifuge for 4 min. Remove 62.5 μ L of the supernatant

and add it to 62.5 μL 50 mM KH_2PO_4 , add 250 μL diethyl ether, vortex for 10 s, centrifuge for 5 min, filter (0.45 μm) the lower aqueous layer, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 75 \times 4.6 TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM

Retention time: 12
Internal standard: trimethoprim (30)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS

serum; comparison with fluorescence polarization immunoassay

REFERENCE

Morishige,H.; Shuto,H.; Ieiri,I.; Otsubo,K.; Oishi,R. Instability of standard calibrators may be involved in over-estimating vancomycin concentrations determined by fluorescence polarization immunoassay, *Ther.Drug Monit.*, **1996**, 18, 80–85.

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 150 \times 5 μm C18 reverse-phase
Mobile phase: MeCN:5 mM KH_2PO_4 22:78
Flow rate: 1.5
Detector: UV 229

CHROMATOGRAM

Retention time: 1.32
Internal standard: salicylic acid (3.94)

KEY WORDS

stability-indicating; ophthalmic solutions

REFERENCE

Tse,M.M.; Liu,C.-M.; Wu,J.; Gee,W.L.; Lin,E.T. HPLC analysis of omeprazole in human plasma (Abstract APQ 1018), *Pharm.Res.*, **1996**, 13, S7.

SAMPLE

Matrix: formulations

Sample preparation: Reconstitute vancomycin injection with water to a drug concentration of 50 mg/mL, dilute with artificial tears solution (Alcon) to a concentration of 31 mg/mL. Dilute 1000-fold with water and inject a 200 μL aliquot.

HPLC VARIABLES

Column: Microsorb MV C18 (Rainin)
Mobile phase: MeCN:5 mM pH 2.8 Na_3PO_4 78:22
Flow rate: 1.5
Injection volume: 200
Detector: UV 229

CHROMATOGRAM

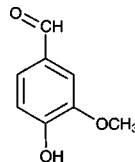
Retention time: 2.3

OTHER SUBSTANCES**Simultaneous:** degradation products**KEY WORDS**

ophthalmic solution; artificial tears; powder; stability-indicating

REFERENCEFuhrman, L.C., Jr.; Stroman, R.T. Stability of vancomycin in an extemporaneously compounded ophthalmic solution, *Am. J. Health-Syst. Pharm.*, **1998**, 55, 1386–1388.

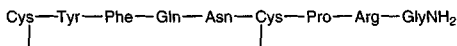
Vanillin

Molecular formula: C₈H₈O₃**Molecular weight:** 152.15**CAS Registry No.:** 121-33-5**Merck Index:** 10069**SAMPLE****Matrix:** solutions**Sample preparation:** Inject a 10 µL aliquot of a solution in MeOH:water 50:50.**HPLC VARIABLES****Column:** 150 × 3.9 4 µm Novapack C18**Mobile phase:** Gradient. A was 0.5% formic acid in water. B was 0.5% formic acid in MeOH. A: B 95:5 for 5 min, to 70:30 over 15 min (Waters curve 9), to 60:40 over 10 min (Waters curve 9), to 55:45 over 10 min (Waters curve 9), to 50:50 over 10 min (Waters curve 9) (*J. Chromatogr. A*, 1994, 683, 31).**Flow rate:** 1**Injection volume:** 10**Detector:** F following post-column reaction. The column effluent flowed through a 10 m × 0.33 mm ID coil of PTFE tubing irradiated with five 8 w low-pressure mercury lamp to the detector. The design of the photoreactor is detailed in the paper.**CHROMATOGRAM****Retention time:** 4.5 (protocatechualdehyde), 6.5 (2,5-dihydroxybenzaldehyde), 7.5 (4-hydroxybenzaldehyde), 8.5 (3-hydroxybenzaldehyde), 12.5 (salicaldehyde), 15 (vanillin), 17 (isovanillin), 24 (o-vanillin), 31.5 (m-anisaldehyde), 40 (veratraldehyde), 42.5 (2,4-dimethoxybenzaldehyde), 43.5 (3,5-dimethoxybenzaldehyde)**KEY WORDS**

post-column photochemical derivatization

REFERENCELores, M.; Garcia, C.M.; Cela, R. Selectable-power photoreactor for flow-injection analysis systems and high-performance liquid chromatography post-column photochemical derivatization, *J. Chromatogr. A*, **1996**, 724, 55–65.

Vasopressin



Molecular formula: C₄₆H₆₅N₁₅O₁₂S₂

Molecular weight: 1084.25

CAS Registry No.: 9034-50-8

Merck Index: 10073

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μ L MeOH: buffer 50:50, inject a 5-75 μ L aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]

HPLC VARIABLES

Column: 250 \times 2.5 μ m Ultrasphere octyl

Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min, maintain at 70:30 for 1 h. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)

Column temperature: 60

Flow rate: 0.3

Injection volume: 5-75

Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m \times 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM

Retention time: 17

Limit of detection: 100 fmole

OTHER SUBSTANCES

Extracted: adrenocorticotropin, angiotensin I, angiotensin II, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, gonadorelin (LHRH), somatoliberin

KEY WORDS

plasma; SPE; post-column reaction

REFERENCE

Rhodes, G.R.; Boppana, V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J. Chromatogr.*, **1988**, *444*, 123-131.

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Bond Elut C8 SPE cartridge with 4 mL MeOH and 4 mL water, do not allow to run dry. Immediately after preparation add 12 μ L 8 M acetic acid to each 1 mL of plasma. 1 mL Acidified plasma + 4 mL 100 mM HCl, add to the SPE cartridge, wash with 4 mL MeCN:0.1% trifluoroacetic acid 10:90, elute with 1.5 mL MeCN:0.1% trifluoroacetic acid 60:40. Evaporate the eluate to dryness under reduced pressure, reconstitute with 10 mM ammonium acetate containing 0.1% bovine serum albumin, inject a 500 μ L aliquot (of pooled extracts).

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: Gradient. MeOH:10 mM pH 4.15 ammonium acetate from 15:85 to 55:45 over 40 (?) min
Flow rate: 1
Injection volume: 500
Detector: UV 210

CHROMATOGRAM
Retention time: 23

KEY WORDS
rat; plasma; SPE

REFERENCE
Van de Heijning,B.J.M.; Koekkoek-van den Herik,I.; Iványi,T.; Van Wimersma Greidanus,T.B. Solid-phase extraction of plasma vasopressin: evaluation, validation and application, *J.Chromatogr.*, **1991**, 565, 159–171.

SAMPLE
Matrix: blood, tissue
Sample preparation: Condition a Sep-Pak ODS SPE cartridge with MeOH. Homogenize 500 mg tissue with 6 mL 100 mM pH 7.4 Tris buffer. Acidify a 2 mL aliquot of plasma or tissue homogenate with 200 μ L 1 M HCl, add to the SPE cartridge, elute with 3 mL MeOH over 3 min, elute with 2 mL over 1 min. Evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue in 200 μ L mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES
Column: 280 \times 5 Dynamax 300-A C8 (Rainin)
Mobile phase: MeCN:water 20:80 containing 0.1% trifluoroacetic acid, 50 mM heptanesulfonic acid and 30 mM triethylamine, pH adjusted to 2.5 with Na₂HPO₄
Flow rate: 1
Injection volume: 20
Detector: UV 200-400

CHROMATOGRAM
Retention time: 6.89 (arginine vasopressin)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: lypressin, oxytocin

KEY WORDS
pig; plasma; SPE; heart

REFERENCE
Rao,P.S.; Weinstein,G.S.; Wilson,D.W.; Rujikarn,N.; Tyras,D.H. Isocratic high-performance liquid chromatography-photodiode-array detection method for determination of lysine- and arginine-vasopressins and oxytocin in biological samples, *J.Chromatogr.*, **1991**, 536, 137–142.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10-20 μ L aliquot of a 500 μ g/mL solution in water.

HPLC VARIABLES
Column: 250 \times 5 10 μ m Nucleosil 10 C18
Mobile phase: MeOH:50 mM pH 6.5 ammonium acetate 39:61
Flow rate: 2
Injection volume: 10-20
Detector: UV 220

OTHER SUBSTANCES
Simultaneous: vasopressin analogs

REFERENCE

Lindeberg, G. Separation of vasopressin analogues by reversed-phase high-performance liquid chromatography, *J. Chromatogr.*, **1980**, *193*, 427–431.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 5 Nova-Pak C18

Mobile phase: Gradient. MeCN:10 mM pH 5.0 acetate buffer 15:85 for 10 min, then to 18:82 over 5 min, maintain at 18:82 for 25 min.

Flow rate: 1

Detector: UV 214, UV 280

CHROMATOGRAM

Retention time: 9.9 (arginine vasopressin)

OTHER SUBSTANCES

Simultaneous: lysipressin, oxytocin

KEY WORDS

hippopotamus

REFERENCE

Rouille, Y.; Chauvet, M.T.; Chauvet, J.; Acher, R.; Hadley, M.E. The distribution of lysine vasopressin (lysipressin) in placental mammals: a reinvestigation of the Hippopotamidae (*Hippopotamus amphibius*) and Tayassuidae (*Tayassu angulatus*) families, *Gen. Comp. Endocrinol.*, **1988**, *71*, 475–483.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Kromasil (Alltech)

Mobile phase: MeCN:pH 7 phosphate buffer 20:80

Flow rate: 2

Detector: UV 254

CHROMATOGRAM

Retention time: 3 (8-arginine vasopressin)

OTHER SUBSTANCES

Simultaneous: oxytocin

REFERENCE

Supelco Catalog, **1993**, p. 525.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 mL water and 3 mL MeCN (in this order ?). Homogenize 400 mg brain tissue with 2 mL 100 mM HCl, add 20 μL 10 μM IS, add 2 mL acetone, mix, centrifuge at 2450 g for 15 min. Remove the supernatant and add it to 220 μL 1 M sodium bicarbonate and 500 μL 100 mM disodium EDTA, centrifuge at 2450 g for 15 min. Remove the supernatant and evaporate it to remove the acetone, dilute the aqueous residue with 2 mL water, add to the SPE cartridge. wash with 1 mL water, wash with 3 mL 100 mM HCl, wash with two 3 mL portions of dichloromethane, wash with 1 mL water, wash with 2 mL 100 mM pH 8.0 phosphate buffer, wash with 2 mL water, elute with 2 mL MeCN:100 mM pH 2.3 phosphate buffer 70:30. Evaporate the eluate under reduced pressure, make up to 400 μL with water, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 200 × 4.5 µm TSKgel ODS-120T (Tosoh)

Mobile phase: Gradient. A was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 1:20:79. B was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 60:20:20. A:B from 90:10 to 55:45 over 33 min, maintain at 55:45 for 7 min, to 0:100 (step gradient), maintain at 0:100.

Flow rate: 1

Injection volume: 100

Detector: F ex 325 em 435 following post-column reaction. The column effluent mixed with 2 mM benzoin in 1.6 M KOH containing 700 mM 2-mercaptoethanol and this mixture flowed through a 15 m × 0.33 mm ID PTFE coil at 76 ± 1°. The effluent from this coil mixed with 500 mM Tris containing 2.1 M HCl pumped at 0.4 mL/min and this mixture flowed to the detector.

CHROMATOGRAM

Retention time: 19.1

Internal standard: [D-Phe¹¹]-neurotensin (40.0)

Limit of detection: 4.4 pmole

OTHER SUBSTANCES

Extracted: bradykinin, dynorphin 1-8, gonadorelin, kallidin, leucine enkephalin-Arg, methionine enkephalin-Arg-Gly-Leu, methionine enkephalin-Arg-Phe, α-neoendorphin, β-neoendorphin, neurotensin, substance P

KEY WORDS

post-column reaction; rat; brain; SPE

REFERENCE

Ohno, M.; Kai, M.; Ohkura, Y. High-performance liquid chromatographic determination of substance P-like arginine-containing peptide in rat brain by on-line post-column fluorescence derivatization with benzoin, *J. Chromatogr.*, **1989**, *490*, 301–310.

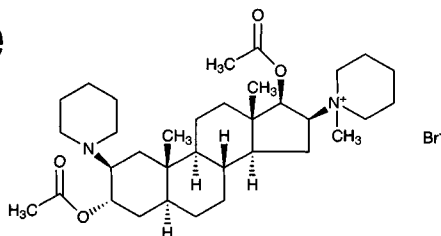
Vecuronium bromide

Molecular formula: C₃₄H₅₇BrN₂O₄

Molecular weight: 637.74

CAS Registry No.: 50700-72-6

Merck Index: 10075

**SAMPLE**

Matrix: bile, blood, perfusate, tissue, urine

Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL MeOH:MeCN 2:1 and 1 mL water. Acidify 1 mL plasma with 150 µL 1 M NaH₂PO₄. Homogenize liver, centrifuge. Dilute 1 µL urine or bile to 1 mL with 100 mM pH 3 NaH₂PO₄. Add 20–200 ng IS to 1 mL acidified plasma, liver homogenate supernatant, diluted urine, diluted bile, or liver perfusate, add to the SPE cartridge, wash with 1 mL water, wash with 1 mL 100 mM pH 3 NaH₂PO₄, elute with 400 µL mobile phase, discard first 100 µL eluate, inject a 200 µL aliquot of the remaining eluate.

HPLC VARIABLES

Column: 150 × 3.9 µm Nova-Pak C18

Mobile phase: Dioxane:water 20:80 containing 100 mM NaH₂PO₄ and 0.44 mM 9,10-dimethoxyanthracene-2-sulfonate, pH adjusted to 3 with phosphoric acid. (Caution! Dioxane is a carcinogen!) (After each series of analyses flush column with 200 mL MeOH then re-equilibrate with 120 mL mobile phase.)

Flow rate: 1

Injection volume: 200

Detector: F ex 380 em 452 following post-column extraction. The column effluent mixed with dichloroethane pumped at 1.6 mL/min and the mixture flowed through a 1 m \times 0.25 mm i.d. stainless steel coil to a phase separator (Anal. Chim. Acta 1987, 192, 267) then the organic phase flowed through the detector.

CHROMATOGRAM

Retention time: 13

Internal standard: 1-(3 α ,17 β -diacetoxy-2 β -piperidino-5 α -androstan-16 β ,5 α -yl)piperidine (16)

Limit of detection: 5 ng/mL

KEY WORDS

SPE; pharmacokinetics; rat; dog; human; cat; plasma; liver; post-column reaction; post-column extraction

REFERENCE

Paanakker, J.E.; Thio, J.M.; Van den Wildenberg, H.M.; Kaspersen, F.M. Assay of vecuronium in plasma using solid-phase extraction, high-performance liquid chromatography and post-column ion-pair extraction with fluorimetric detection, *J. Chromatogr.*, **1987**, 421, 327–335.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 250 μ L picric acid (1:10 dilution of saturated picric acid solution) + 250 μ L pancuronium solution + 250 μ L water + 5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150–250 μ L MeCN: water 40:60, centrifuge at 1500 g for 4 min, inject a 20–100 μ L aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 μ Porasil

Mobile phase: MeCN:2 mM sulfuric acid 50:50

Flow rate: 2

Injection volume: 20–100

Detector: conductivity 2500 nS full scale

CHROMATOGRAM

Retention time: 4.7

Internal standard: pancuronium (5.7)

Limit of detection: 10 ng/mL

KEY WORDS

plasma

REFERENCE

Bjorksten, A.R.; Beemer, G.H.; Crankshaw, D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J. Chromatogr.*, **1990**, 533, 241–247.

SAMPLE

Matrix: blood

Sample preparation: 10 mL Blood + 1 mL 1 M pH 2.5 KH_2PO_4 + 50 μ L 6 μ g/mL d-tubocurarine + 1 mL 3% perchloric acid + 12 mL dichloromethane, rotate for 20 min, centrifuge at 1520 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μ L mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: 25 mm long CN guard column

Column: 250 \times 4.6 Spherisorb S5 CN

Mobile phase: MeCN:100 mM pH 5 phosphate 50:50

Column temperature: 40

Flow rate: 1.5

Detector: UV 214 or E, BAS LC-4B, LC-17A thin-layer flow cell, working glassy carbon electrode (W1) +0.65 V, quantitating glassy carbon electrode (W2) +1.05 V, Ag/AgCl reference electrode and auxiliary electrode

CHROMATOGRAM

Retention time: 1 (relative retention time)

Internal standard: d-tubocurarine (relative retention time = 0.5)

Limit of detection: 3 ng/mL

OTHER SUBSTANCES

Noninterfering: atropine, apresoline, haloperidol, fentanyl, labetalol, thiopental, atracurium, diazepam

REFERENCE

Hu, O.Y.; Chou, C.H.; Ho, W.; Ho, S.T. Determination of vecuronium in blood by HPLC with UV and electrochemical detection: a pilot study in man, *Proc. Natl. Sci. Coun. Repub. China [B]*, **1991**, 15, 186–190.

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut C1 SPE cartridge with two 3 mL portions of MeOH and two 3 mL portions of water. Add 1 mL plasma, 1 mL water, and 100 μ L 15 μ g/mL IS in 100 mM pH 5.0 ($\text{NH}_4\text{H}_2\text{PO}_4$) to the SPE cartridge. Wash with 3 mL water, wash with 3 mL MeCN, wash with 3 mL MeOH, elute with two 500 μ L portions of 10 mM sodium perchlorate in MeOH under gravity for 3 min then under vacuum. Evaporate the eluate to dryness under vacuum, reconstitute the residue in 100 μ L mobile phase, inject a 30 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.6 5 μ m Spherisorb CN

Mobile phase: MeCN:33 mM phosphoric acid 40:60, adjusted to pH 5.55 with ammonium hydroxide

Flow rate: 2

Injection volume: 30

Detector: E, Environmental Science Associates Coulochem 5100A, 5010 analytical cell, screen electrode +0.4 V (detector 1), working electrode +0.8 V (detector 2)

CHROMATOGRAM

Retention time: 7.3

Internal standard: Org 7465 (dipropionyl ester of vecuronium) (9.8)

Limit of quantitation: 3.9 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

plasma; SPE

REFERENCE

Ducharme, J.; Varin, F.; Bevan, D.R.; Donati, F.; Théorêt, Y. High-performance liquid chromatography-electrochemical detection of vecuronium and its metabolites in human plasma, *J. Chromatogr.*, **1992**, 573, 79–86.

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 0.5% solution in the mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 5 μ m SI 100 (Bio Separation Technologies)

Mobile phase: MeCN:100 mM sodium perchlorate 96:4

Flow rate: 1

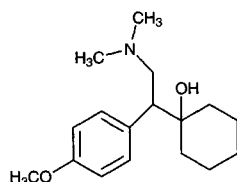
Injection volume: 20

Detector: UV 213

CHROMATOGRAM**Retention time:** 4.2**OTHER SUBSTANCES****Simultaneous:** pipecuronium**Interfering:** pancuronium**REFERENCE**

Gazdag,M.; Babják,M.; Kemenes-Bakos,P.; Görög,S. Analysis of steroids. XLI. Ion-pair high-performance liquid chromatographic separation of quaternary ammonium steroids on silica, *J.Chromatogr.*, **1991**, 550, 639–644.

Venlafaxine

Molecular formula: $C_{17}H_{27}NO_2$ **Molecular weight:** 277.41**CAS Registry No.:** 93413-69-5, 99300-78-4 (HCl)**Merck Index:** 10079**Lednicer No.:** 4 26**SAMPLE****Matrix:** blood

Sample preparation: Mix 50 μ L 100 ng/mL maprotiline with 1 mL plasma and 400 μ L 700 mM pH 9.7 bicarbonate buffer, vortex for 1 min, add 1.5 mL isoamyl alcohol:hexane 7.5:92.5, shake at 300 rpm for 30 min. Centrifuge at 1500 g for 15 min, freeze at -20° overnight, decant the organic layer into a tube containing 200 μ L 0.05% orthophosphoric acid. Shake vigorously at 300 rpm for 45 min, centrifuge at 1500 g for 15 min, inject a 100 μ L aliquot of the aqueous phase.

HPLC VARIABLES**Guard column:** 13 \times 4.3 5 μ m C4/E butyl-bonded reversed-phase (MetaChem Technologies)**Column:** 150 \times 4.6 5 μ m C4/E butyl-bonded reversed-phase(MetaChem Technologies)**Mobile phase:** MeCN:40 mM pH 6.8 sodium phosphate buffer 50:50**Flow rate:** 1.5**Injection volume:** 100**Detector:** F ex 276 em 598**CHROMATOGRAM****Retention time:** 7**Internal standard:** maprotiline (10)**Limit of detection:** 1 ng/mL**OTHER SUBSTANCES****Extracted:** metabolites**KEY WORDS**

plasma

REFERENCE

Vu,R.L.; Helmeste,D.; Albers,L.; Reist,C. Rapid determination of venlafaxine and O-desmethylvenlafaxine in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1997**, 703, 195–201.

SAMPLE**Matrix:** blood

Sample preparation: Condition a 50 mg Carboxymethyl Isolute SPE cartridge with 1 mL MeOH and 1 mL 25 mM pH 6.8 phosphate buffer, dry under vacuum. 500 μ L Plasma + 20 ng IS, add

to the SPE cartridge, wash with two 1 mL portions of 25 mM pH 6.8 phosphate buffer, dry under vacuum, elute with 1 mL 1% ammonia in MeOH, evaporate to dryness under vacuum at 40°, reconstitute the residue in 100 µL MeOH, vortex, inject a 25 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb ODS/CN

Mobile phase: MeOH:50 mM pH 4.8 potassium phosphate buffer 70:30

Flow rate: 1

Injection volume: 25

Detector: E, ESA, Model 5100 A, Model 5010 analytical cell +650 mV on channel 1, +950 mV on channel 2, Model 5020 guard cell +980 mV

CHROMATOGRAM

Retention time: 8.8

Internal standard: paroxetine (9.6), desipramine (11.5)

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Interfering: m-chlorophenylpiperazine

KEY WORDS

plasma; SPE

REFERENCE

Clement,E.M.; Odontiadis,J.; Franklin,M. Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine, in human plasma by high-performance liquid chromatography with coulometric detection and utilisation of solid-phase extraction, *J.Chromatogr.B*, **1998**, 705, 303–308.

Verapamil

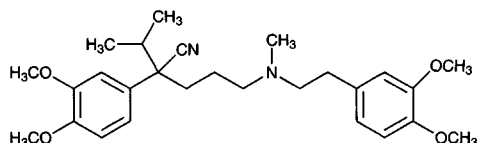
Molecular formula: C₂₇H₃₈N₂O₄

Molecular weight: 454.61

CAS Registry No.: 52-53-9, 152-11-4 (HCl)

Merck Index: 10083

Lednicer No.: 4 34



SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 µL 2 M NaOH + IS, extract with 5 mL pentane: dichloromethane 2:1. Remove the organic layer and evaporate it to dryness under a gentle stream of nitrogen, reconstitute the residue in mobile phase, inject a 1 µL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 10 µm Chiralpak AD (Chiral Technologies, Exton, PA)

Mobile phase: n-Hexane:isopropanol:diethylamine 92.5:7.5:0.1

Column temperature: 32

Flow rate: 0.2

Injection volume: 1

Detector: MS, SCIEX API 300 tandem mass, positive ion mode, nebulizer 440°, scan 455.0/165.0

CHROMATOGRAM

Retention time: 4.04 (S), 4.75 (R)

Internal standard: (+)-glaucine (5.49)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; chiral; small-bore

REFERENCE

Alebic-Kolbah,T.; Zavitsanos,A.P. Chiral bioanalysis by normal phase high-performance liquid chromatography-atmospheric pressure ionization tandem mass spectrometry, *J.Chromatogr.A*, **1997**, 759, 65-77.

SAMPLE

Matrix: blood

Sample preparation: Condition column A with two 995 μ L portions of mobile phase at 3 mL/min and then with 995 μ L solution B. Wash the donor channel of the dialyzer (Gilson ASTED XL fitted with a Cuprophane cellulose acetate membrane with a molecular mass cut-off of 15000) with 2 mL solution A at 3 mL/min and wash the acceptor channel with 2 mL solution B. Add 50 μ L 1.2 μ g/mL gallopamil in water to 450 μ L plasma, mix by bubbling. Dialyze a 370 μ L aliquot against 9 mL solution B is pumped through the acceptor channel at 1 mL/min. Pass the dialysate through column A, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B. (Solution A was 10 mM pH 3 acetate buffer containing 0.01% Triton X-100 and 50 μ g/mL sodium azide. Solution B was 10 mM pH 3 acetate buffer containing 50 μ g/mL sodium azide.)

HPLC VARIABLES

Column: A 30 μ m Nucleosil CN; B 5 μ m LiChrospher 100 RP-18 guard column + 4 μ m Superspher 100 RP-18

Mobile phase: MeCN:2-aminoheptane:buffer 25:0.5:75 (Buffer was 10 mM sodium acetate adjusted to pH 3.0 with acetic acid.)

Column temperature: 35

Flow rate: 0.9

Detector: F ex 275 em 310

CHROMATOGRAM

Retention time: 12.5

Internal standard: gallopamil (15)

Limit of detection: 1.3 ng/mL

Limit of quantitation: 4.3 ng/mL

OTHER SUBSTANCES

Extracted: norverapamil

KEY WORDS

dialysate; column-switching; plasma

REFERENCE

Ceccato,A.; Chiap,P.; Hubert,P.; Toussaint,B.; Crommen,J. Automated determination of verapamil and norverapamil in human plasma with on-line coupling of dialysis to high-performance liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, 750, 351-360.

SAMPLE

Matrix: blood

Sample preparation: Mix 500 μ L plasma with 100 μ L 200 ng/mL R-(+)-propranolol in mobile phase, 100 μ L 1 M NaOH, and 5 mL diethyl ether. Shake for 10 min and centrifuge at 1000 g for 10 min. Evaporate 4 mL of the diethyl ether layer to dryness under nitrogen at 40°, reconstitute the residue with 100 μ L mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Chiralpak AD (Daicel)

Mobile phase: Hexane:2-propanol:diethylamine 93.9:6:0.1

Flow rate: 1.2

Injection volume: 50

Detector: F ex 272 em 312

CHROMATOGRAM

Retention time: 10 (S-(-)), 12 (R-(+))

Internal standard: R-(+)-propranolol (7.8)

Limit of detection: 500 pg/mL (S-(-)), 1.3 ng/mL (R-(+))

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

chiral; pharmacokinetics; plasma

REFERENCE

Hashiguchi,M.; Ogata,H.; Maeda,A.; Hirashima,Y.; Ishii,S.; Mori,Y.; Amamoto,T.; Handa,T.; Otsuka,N.; Irie,S.; Urae,A.; Urae,R.; Kimura,R. No effect of high-protein food on the stereoselective bioavailability and pharmacokinetics of verapamil, *J.Clin.Pharmacol.*, **1996**, 36, 1022–1028.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 \times 4.6 5 μ m Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10–30

Detector: UV 201.7

CHROMATOGRAM

Retention time: 15.365

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149–163.

SAMPLE

Matrix: cell culture media, microsomal incubations

Sample preparation: 250 μ L Sample + 20 μ L 50 μ g/mL IS + 6 mL diethyl ether, vortex in shaker for 20 min, centrifuge at 4000 g for 20 min. Remove a 5 mL aliquot of the organic layer, add 250 μ L 100 mM HCl, vortex in an overhead shaker for 20 min, centrifuge at 4000 g for 20 min, discard the organic phase, inject a 40 μ L of the aqueous phase.

HPLC VARIABLES

Column: 150 \times 8 4 μ m Nucleosil 100-3 C8

Mobile phase: MeCN:MeOH:buffer 28:5:67, adjusted to pH 4.2 with 1 M HCl (Prepare the buffer by dissolving 0.71 g NaH_2PO_4 and 2 g 1-heptanesulfonic acid sodium salt in 1 L water.)

Column temperature: 51

Flow rate: 1.7

Injection volume: 40
Detector: F ex 280 em 310

CHROMATOGRAM

Retention time: 19
Internal standard: norverapamil (7)
Limit of quantitation: 200 nM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

liver

REFERENCE

Fischer,U.; Wacke,R.; Stange,J.; Nitschke,F.-P.; Adam,U.; Drewelow,B. Rapid HPLC assay for verapamil and its metabolites: use for application to in vitro studies, *Pharmazie*, **1996**, *51*, 220–223.

SAMPLE

Matrix: cells

Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2×10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 7 μm Hibar LiChrocart RP 18 (Merck)

Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 237

CHROMATOGRAM

Retention time: 12.4

Internal standard: altretamine (6.2)

Limit of detection: 8 pmol

Limit of quantitation: 27 pmol

OTHER SUBSTANCES

Extracted: daunorubicin, doxorubicin, vincristine, S 9788

KEY WORDS

human; cells; epidermoid carcinoma

REFERENCE

Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.

SAMPLE

Matrix: hepatocyte cultures, microsomal incubations

Sample preparation: Add 20 μL 50 $\mu\text{g/mL}$ IS and 6 mL diethyl ether to 250 μL hepatocyte culture or microsomal incubation, shake for 20 min, centrifuge at 4000 g for 20 min. Remove 5 mL of the organic phase and add it to 250 μL 100 mM HCl. Shake for 20 min and centrifuge at 4000 g for 20 min. Inject a 40 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 150 \times 8 4 μm Nucleosil 100-3 C8

Mobile phase: MeCN:MeOH:buffer 28:5:67, adjusted to pH 4.2 with 1 M HCl (Buffer was 710 mg sodium dihydrogen phosphate and 2.0 g 1-heptanesulfonic acid sodium salt in 1 L distilled water.)

Column temperature: 51

Flow rate: 1.7

Injection volume: 40

Detector: F ex 280 em 310

CHROMATOGRAM

Retention time: 18

Internal standard: D 600 (Knoll AG, Germany) (16.5)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

liver; rat

REFERENCE

Fischer,U.; Rohde,B.; Wacke,R.; Stange,J.; Nitschke,F.P.; Adam,U.; Drewelow,B. Prediction of in vivo drug interaction from in vitro systems exemplified by interaction between verapamil and cimetidine using human liver microsomes and primary hepatocytes, *J.Clin.Pharmacol.*, **1997**, 37, 1150–1159.

SAMPLE

Matrix: perfusate

Sample preparation: Dilute perfusate with mobile phase, inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 10 \times 3 Chiral AGP (Chromotech, Sweden)

Column: 150 \times 4.0 Chiral AGP (Chromotech, Sweden)

Mobile phase: MeCN: pH 7.6 phosphate buffer (I=0.01) 22:78

Column temperature: 30

Flow rate: 1

Injection volume: 50

Detector: F ex 232 em 310

CHROMATOGRAM

Limit of detection: 2.9 ng/mL

Limit of quantitation: 5.5 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

chiral; intestine

REFERENCE

Sandström,R.; Karlsson,A.; Knutson,L.; Lennernäs,H. Jejunal absorption and metabolism of R/S-verapamil in humans, *Pharm.Res.*, **1998**, 15, 856–862.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve a sample in water, mix a 1 mL aliquot with 100 μ L 13.36 μ g/mL IS, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 4.0 5 μ m Chiral-AGP (Baker)

Mobile phase: MeCN:pH 6.8 (I=0.01) ammonium acetate 11:89 (Buffer was adjusted to pH 6.8 with ammonium hydroxide or acetic acid.)

Column temperature: 22

Flow rate: 0.9

Injection volume: 20

Detector: UV 225; MS, Finnigan MAT SSQ 710A, interface particle beam, desolvation chamber 45°, nebulizing gas helium, electron impact mode 70 eV, source 250°, filament current 200 μ A, electron multiplier 1500 V, m/z 45-400

CHROMATOGRAM

Retention time: 9.74 ((2R)-(+)), 12.02 ((2S)-(-))

Internal standard: procaine hydrochloride (5.09)

Limit of detection: 109 ng/mL ((2R)-(+)), 114 ng/mL ((2S)-(-))

OTHER SUBSTANCES

Also analyzed: gallopamil

KEY WORDS

chiral

REFERENCE

Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477-483.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 4.6 5 μ m Spherisorb SCX

Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid

Flow rate: 1

Injection volume: 1-10

Detector: UV 270

CHROMATOGRAM

Retention time: 7.8

OTHER SUBSTANCES

Simultaneous: cimetidine, clomipramine, halofantrine, haloperidol, minoxidil, reserpine

REFERENCE

Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335-341.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve a sample in water, inject an aliquot.

HPLC VARIABLES

Column: 100 \times 4.0 5 μ m Chiral-AGP (Baker)

Mobile phase: Gradient. A was MeCN. B was isopropanol. C was pH 6.8 (I = 0.01) ammonium acetate adjusted to pH 6.8 with ammonium hydroxide or acetic acid. A:B:C from 11:1:88 to 7:1:92 over 1.5 min, maintain at 7:1:92 for 35 min, to 11:1:88 over 5 min

Column temperature: 22

Flow rate: 0.9

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 14 ((2R)-(+)), 19 ((2S)-(-))

OTHER SUBSTANCES

Simultaneous: gallopamil

KEY WORDS

chiral

REFERENCE

Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477–483.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot of a 100–500 $\mu\text{g/mL}$ solution in mobile phase.

HPLC VARIABLES

Column: 100 \times 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60

Flow rate: 0.5–2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 10.96

OTHER SUBSTANCES

Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyrindamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid

Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE

Matrix: urine

Sample preparation: Condition a 7 mm/3 mL 3M Empore C8 SPE disc (Varian Associates, CA) with 500 μL MeOH and two 500 μL portions of water. Mix 500 μL urine, 50 μL 400 ng/mL IS in MeOH:water 50:50, and 500 μL 30 mM pH 10 phosphate buffer. Add to the SPE disc. Wash twice with 500 μL water, twice with 500 μL MeCN:water 30:70, elute with two 500 μL portions of MeOH, apply full vacuum for a few seconds. Evaporate to dryness at 60°. Reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: NewGuard C18

Column: 250 \times 4.6 10 μm Chiralcel OD-R CSP

Mobile phase: MeCN:200 mM sodium perchlorate 40:60

Flow rate: 0.8

Injection volume: 100

Detector: F ex 230 em 312

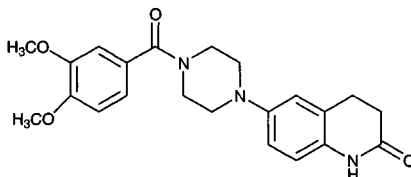
CHROMATOGRAM**Retention time:** 21.8 (R-), 24.2 (S-)**Internal standard:** (+)-glaucine (16.5)**Limit of quantitation:** 3 ng/mL**OTHER SUBSTANCES****Extracted:** metabolites**KEY WORDS**

chiral; SPE

REFERENCE

Asafu-Adjaye, E.B.; Shiu, G.K. Solid-phase extraction-high-performance liquid chromatography determination of verapamil and norverapamil enantiomers in urine, *J. Chromatogr. B*, **1998**, 707, 161–167.

Vesnarinone

Molecular formula: C₂₂H₂₅N₃O₄**Molecular weight:** 395.46**CAS Registry No.:** 81840-15-3**Merck Index:** 10105**Lednicer No.:** 5 122**SAMPLE****Matrix:** blood, microsomal incubations, urine

Sample preparation: Condition a 3 mL 200 mg UCT C18 (United Chemical Technologies, Bristol PA) SPE cartridge with 2 mL MeOH, 2 mL 10 mM ascorbic acid, and 2 mL 80 mM ammonium acetate solution. 500 µL Microsomal incubation + 500 µL MeOH, mix, centrifuge at 9500 g for 10 min. Add 100 µL supernatant from the microsomal incubation, 500 µL plasma, or 50 µL urine to 50 µL MeOH and 50 µL 1 µg/mL IS solution, vortex, add 1 mL 50 mM ammonium acetate, vortex for 30 s, centrifuge at 3000 rpm for 10 min. Add the mixture to the SPE cartridge, wash with 2 mL 50 mM ammonium acetate and 2 mL n-butyl chloride. Dry the cartridge under a light vacuum, elute with three 1 mL portions of MeOH:6 M acetic acid:10 mM ascorbic acid 95:2:3. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue with 500 µL mobile phase, inject an aliquot.

HPLC VARIABLES**Column:** 45 × 4.6 5 µm Ultrasphere ODS**Mobile phase:** Gradient. A was MeCN:0.1% acetic acid 2.5:97.5. B was MeCN:0.1% acetic acid 80:20. A:B from 100:0 to 0:100 over 7 min.**Detector:** MS, PE Sciex API III, turbo ion spray interface in MS/MS mode, liquid nitrogen nebulizer auxiliary gas, ultrahigh purity nitrogen, argon curtain gas and collision gas**CHROMATOGRAM****Internal standard:** OPC-8192 (Otsuka Pharmaceutical, Japan)**Limit of detection:** 200 ng/mL (plasma), 500 ng/mL (urine)**OTHER SUBSTANCES****Extracted:** metabolites**KEY WORDS**

plasma; SPE; pharmacokinetics

REFERENCE

Wandel, C.; Lang, C.C.; Cowart, D.C.; Girard, A.F.; Bramer, S.; Flockhart, D.A.; Wood, A.J.J. Effect of CYP3A inhibition on vesnarinone metabolism in humans, *Clin. Pharmacol. Ther.*, **1998**, 63, 506–511.

Vidarabine

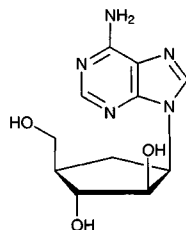
Molecular formula: $C_{10}H_{13}N_5O_4$

Molecular weight: 267.24

CAS Registry No.: 5536-17-4, 24356-66-9 (monohydrate), 29984-33-6 (phosphate), 71002-10-3 (sodium phosphate)

Merck Index: 10113

Lednicer No.: 4 122



SAMPLE

Matrix: aqueous humor, blood, urine

Sample preparation: Plasma. Filter (Amicon CF25 or CF50A Centrifo membrane cones) plasma while centrifuging at 100 g for 1 h. 160 μ L Ultrafiltrate + 20 μ L 20 μ g/mL IS in water + 20 μ L 100 mM pH 6.3 sodium acetate buffer, mix, inject a 10 μ L aliquot. Urine. Filter (Schleicher and Schüll paper No. 497) urine, dilute the filtrate with water, inject an aliquot. Aqueous humor. Inject directly.

HPLC VARIABLES

Column: 150 \times 3.7 Aminex A-28 (equilibrate in 2 M sodium acetate for 24 h before use) (Bio-Rad)

Mobile phase: 7.5 mM pH 6.4 Sodium borate buffer containing 2.5 mM sodium acetate

Column temperature: 60

Flow rate: 0.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 6.5

Internal standard: 8-aminoarabinosyladenine (9.0)

OTHER SUBSTANCES

Extracted: metabolites, adenosine, 9- β -D-arabinofuranosylhypoxanthine

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Schneider, H.G.; Glazko, A.J. High-performance liquid chromatography of adenine and hypoxanthine arabinosides, *J. Chromatogr.*, **1977**, *139*, 370-375.

SAMPLE

Matrix: blood

Sample preparation: Prepare plasma rapidly, keep at 4°. Mix 250 μ L plasma and 50 μ L water at 4°, vortex for 5 s, add 1 mL MeCN with vortexing, centrifuge at 4° at 13000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 400 μ L mobile phase, vortex for 30 s, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 45 \times 4.6 5 μ m Ultrasphere Octadecyl

Column: 250 \times 4.6 5 μ m Ultrasphere Octadecyl

Mobile phase: MeCN:buffer 5:95 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 6.0 with 100 mM NaOH.)

Flow rate: 1

Injection volume: 20

Detector: UV 260

CHROMATOGRAM

Retention time: 12

Internal standard: vidarabine

OTHER SUBSTANCES

Extracted: sinefungin

KEY WORDS

rat; plasma; vidarabine is IS

REFERENCE

Tharasse-Bloch,C.; Brasseur,P.; Favennec,L.; Marchand,J. Determination of sinefungin in rat plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, 674, 247-252.

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Filter (Amicon Centriflo CF25) plasma, urine, or CSF while centrifuging at 1100 g for 20 min, inject a 10-20 μ L aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Ultrasphere ODS

Mobile phase: Gradient. A was 10 mM KH_2PO_4 . B was MeOH:10 mM KH_2PO_4 30:70, pH 4.9. A: B from 90:10 to 70:30 over 15 min. (At the end of each day flush column with 20-30 mL B. If column becomes contaminated wash with water, 1 mM phosphoric acid, water, MeOH:water 70:30, and water for 15-30 min.)

Flow rate: 1

Injection volume: 10-20

Detector: UV 254

CHROMATOGRAM

Retention time: 17.24

Limit of detection: 10 pmole (100 μ L injection)

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: adenine, adenosine, arabinosylhypoxanthine, 5-bromodeoxyuridine, deoxyadenosine, deoxycofomycin, deoxycytidine, deoxyguanosine, deoxyinosine, guanosine, hypoxanthine, inosine, N^1 -methyladenosine, thymidine, uric acid

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Agarwal,R.P.; Major,P.P.; Kufe,D.W. Simple and rapid high-performance liquid chromatographic method for analysis of nucleosides in biological fluids, *J.Chromatogr.*, **1982**, 231, 418-424.

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Add pentostatin to blood samples to prevent deamination. Dilute urine 1:10 with water. 200 μ L Serum, CSF, or diluted urine + 20 μ L isoamyl alcohol + 50 μ L chloroform, vortex for 30 s, centrifuge at 20931 g for 10 min. Remove the aqueous layer and add it to 1 mL cold acetone (0°), vortex for 10 s, centrifuge for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μ L water, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m LiChrosorb RP-8

Mobile phase: MeCN:buffer 4:96 (Buffer was 5 mM sodium pentanesulfonate, pH 7.2.)

Column temperature: 40

Flow rate: 1

Detector: UV 250

CHROMATOGRAM

Retention time: 6.65

Limit of detection: 2.5 μ g/mL (urine), 500 ng/mL (serum, CSF)

OTHER SUBSTANCES

Extracted: metabolites, pentostatin

Noninterfering: chlorothiazide, cytosine arabinoside, guanine arabinoside, hydroxyzine, kanamycin, metaproterenol, nystatin, penicillin G, phenobarbital, prednisone, sulfamethoxazole, theophylline, trimethoprim, uracil arabinoside

KEY WORDS

serum

REFERENCE

Bowman,D.B.; Kauffman,R.E. Reversed-phase high-performance liquid chromatographic method to determine vidarabine and hypoxanthine arabinoside in biological fluids, *J.Chromatogr.*, **1982**, 229, 487-491.

SAMPLE

Matrix: blood, urine

Sample preparation: Centrifuge plasma or urine at 1000 g at 4° for 10 min. Add 1 mL supernatant to 1 mL 12% trichloroacetic acid (in an ice bath), mix thoroughly, let stand for 15 min, centrifuge at 4° at 1000 g for 30 min. Remove the supernatant and extract it four times with water-saturated diethyl ether, discard the ether extracts. Remove traces of ether from the aqueous layer by heating it at 65° with intermittent suction, cool. Remove 500 µL of the aqueous layer and add it to 200 µL 40 mM chloroacetaldehyde in 28 mM pH 5.1 sodium acetate buffer. Heat at 80° for 40 min, cool, extract four times with 1 mL portions of water-saturated diethyl ether, discard the ether extracts. Heat the aqueous layer at 65° with intermittent suction to remove traces of ether, cool. Dilute urine preparation 1:9, do not dilute plasma preparations, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 4 µBondapak C18/Corasil

Column: 250 × 4.6 Spherisorb S5 ODSII octadecyl

Mobile phase: Gradient. A was 20 mM sodium tetraborate decahydrate adjusted to pH 7.7 with 4.4 N phosphoric acid. B was MeOH:20 mM sodium tetraborate decahydrate adjusted to pH 7.7 with 4.4 N phosphoric acid 30:70. A:B from 100:0 to 0:100 over 15 min, maintain at 0:100 for 5 min, return to initial conditions, re-equilibrate for 5 min.

Flow rate: 1

Injection volume: 50

Detector: F ex 315 em 415

CHROMATOGRAM

Retention time: 15.2

Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES

Extracted: Ara-AMP, ara-H (UV detection)

KEY WORDS

plasma; derivatization; pharmacokinetics

REFERENCE

McCann,W.P.; Hall,L.M.; Siler,W.; Barton,N.; Whitley,R.J. High-pressure liquid chromatographic methods for determining arabinosyladenine-5'-monophosphate, arabinosyladenine, and arabinosylhypoxanthine in plasma and urine, *Antimicrob.Agents Chemother.*, **1985**, 28, 265-273.

SAMPLE

Matrix: blood, urine

Sample preparation: Filter urine (0.45 µm). Filter plasma (Centrifree Micropartition, Amicon) while centrifuging, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Microsorb C18

Mobile phase: Gradient. MeCN:50 mM pH 4.8 ammonium acetate from 0.5:99.5 to 12:88 over 40 min

Flow rate: 1
Detector: UV 254, UV 280

CHROMATOGRAM

Retention time: 29

OTHER SUBSTANCES

Extracted: ara-H, ara-MAP, ara-DMAP

KEY WORDS

plasma; rat; monkey; pharmacokinetics; ultrafiltrate

REFERENCE

Lambe,C.U.; Resetar,A.ctor,T.; Koszalka,G.W.; Nelson,D.J. Metabolism and pharmacokinetics of the anti-varicella-zoster virus agent 6-dimethylaminopurine arabinoside, *Antimicrob.Agents Chemother.*, **1992**, *36*, 353–360.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 100 mg vidarabine in a little warm water, add 1 mL DMSO, make up to 100 mL with water. Remove a 125 μ L aliquot, add 1.25 mL 100 μ g/mL inosine in water, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES

Guard column: C18 (Brownlee)

Column: 250 \times 4.6 Partisil 10/ODS-3

Mobile phase: MeCN:buffer 5:95 (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.250 ± 0.005 with acetic acid.)

Flow rate: 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 7.5

Internal standard: inosine (6.5)

OTHER SUBSTANCES

Simultaneous: impurities, adenosine, 2'-deoxyadenosine

REFERENCE

Schroeder,W.III; Cupps,T.L.; Townsend,L.B. Quantitative high-performance liquid chromatography of structurally similar nucleosides, *J.Chromatogr.*, **1983**, *254*, 315–321.

SAMPLE

Matrix: fermentation solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Phenomenex C8

Mobile phase: MeCN:MeOH:50 mM $(\text{HN}_4)_2\text{HPO}_4$ 2.5:2.5:95 adjusted to pH 7.4 with phosphoric acid

Flow rate: 1.5

Detector: UV 258

CHROMATOGRAM

Retention time: 9.1

OTHER SUBSTANCES

Extracted: cytosine, coformycin, (8S)-pentostatin, 2'-deoxyguanosine, pentostatin

REFERENCE

Showalter,H.D.H.; Bunge,R.H.; French,J.C.; Hurley,T.R.; Leeds,R.L.; Leja,B.; McDonnell,P.D.; Edmunds,C.R. Improved production of pentostatin and identification of fermentation cometabolites, *J.Antibiot.(Tokyo)*, **1992**, 45, 1914–1918.

SAMPLE

Matrix: formulations

Sample preparation: Dilute 30 μL of the injection to 100 mL with buffer, add 50 μL 10 mg/mL 5-flucytosine in water, inject a 20 μL aliquot. (Buffer contained 1 g/L $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and 0.363 g/L KH_2PO_4 , pH 7.4.)

HPLC VARIABLES

Column: 150 \times 4.6 5 μm Supelcosil LC 18

Mobile phase: 10 mM KH_2PO_4 adjusted to pH 6.8 with 25% KOH

Flow rate: 1

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.5 (vidarabine phosphate)

Internal standard: 5-flucytosine (k' 0.9)

KEY WORDS

injections; water

REFERENCE

Kwee,M.S.L.; Stolk,L.M.L. Formulation of a stable vidarabine phosphate injection, *Pharm.Weekbl.[Sci.]*, **1984**, 6, 101–104.

SAMPLE

Matrix: perfusate

Sample preparation: 1 mL Perfusate (Krebs solution) + 40 μL chloroacetaldehyde + 360 μL buffer, heat at 80° for 40 min, cool on ice, inject an aliquot. (Krebs solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM calcium chloride, 1.2 mM KH_2PO_4 , 1.2 mM magnesium sulfate, 25 mM sodium bicarbonate, and 5.5 mM glucose. Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4 , pH 4.0. (Prepare chloroacetaldehyde as follows. Cautiously add 1 mL concentrated sulfuric acid to 9 mL water (using eye protection and other protective equipment), add to 10 mL chloroacetaldehyde dimethyl acetal, distil slowly and collect the fraction boiling at 80–85° which contains 1–1.15 M chloroacetaldehyde, store at 0° (Anal. Biochem. 1984, 137, 93).)

HPLC VARIABLES

Guard column: 10 \times 4.6 10 μm Ultron N-phenyl (Shinwa, Kyoto)

Column: 150 \times 4.6 5 μm Ultron N-phenyl (Shinwa, Kyoto)

Mobile phase: MeCN:buffer 1.5:98.5, adjusted to pH 4.5 with 2-diethylaminoethanol (Prepare buffer by mixing 400 mL 100 mM citric acid with 245 mL 200 mM Na_2HPO_4 , pH 4.0.)

Flow rate: 1

Detector: F ex 305 em 420

CHROMATOGRAM

Retention time: 17

Internal standard: vidarabine

OTHER SUBSTANCES

Extracted: adenosine, adenosine diphosphate, adenosine monophosphate, adenosine triphosphate

KEY WORDS

derivatization; vidarabine is IS

REFERENCE

Mohri,K.; Takeuchi,K.; Shinozuka,K.; Bjur,R.A.; Westfall,D.P. Simultaneous determination of nerve-induced adenine nucleotides and nucleosides released from rabbit pulmonary artery, *Anal.Biochem.*, **1993**, *210*, 262–267.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 3 Partisil XS 10/25 C8

Mobile phase: MeCN:buffer 5:95 (Buffer was 800 mL 75 mM sodium borate and 200 mL 10 mM (NH₄)H₂PO₄, pH adjusted to 6.03 with boric acid.)

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Simultaneous: metabolites, adenosine, 9-β-D-arabinofuranosylhypoxanthine, 2'-deoxyadenosine, 2'-deoxyinosine, inosine

REFERENCE

Delia,T.J.; Kirt,D.D. Reversed-phase high-performance liquid chromatographic separation of ribosyl, 2'-deoxy-ribosyl and arabinosyl nucleosides of adenine and hypoxanthine, *J.Chromatogr.*, **1982**, *243*, 173–177.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 50 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm Hypersil ODS

Mobile phase: MeOH:buffer 5:95 (Buffer was 50 mM pH 3.0 phosphate containing 0.4 mM sodium 1-heptanesulfonate.)

Column temperature: 40

Flow rate: 1

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 9.4

Internal standard: vidarabine

OTHER SUBSTANCES

Simultaneous: cytarabine, uracil arabinoside

Noninterfering: allopurinol, cephalosporins, ciprofloxacin, diazepam, metoclopramide, mitoxantrone, ondansetron

KEY WORDS

vidarabine is IS

REFERENCE

Burk,M.; Volmer,M.; Fartash,K.; Schneider,W. Ion-pair liquid chromatography of cytarabine and uracil-arabinoside in human plasma, *Arzneimittelforschung*, **1995**, *45*, 616–619.